The Role of Production Stressors on Gut Health

Gerry Huff, Research Microbiologist
USDA/ARS Poultry Production and Product Safety Research Unit
Fayetteville, AR

TAKE-HOME MESSAGE

The everyday stressors so common to intensive animal production can be detrimental to gut health and can lead to decreases in performance, particularly feed conversion efficiency. Antibiotic growth promoters (AGP's) have been a tool that has enabled production efficiency in stressful environments. In the absence of AGP's, food animal producers will need to decrease production stressors and find alternatives to antibiotics. Yeast feed additives have been shown to enhance gut development and prevent production losses in *Escherichia coli* challenges combined with both cold stress and transport stress in turkeys.

What is Stress? The word 'Stress' has many meanings and symbolizes different things to different people. "Stress" was originally an engineering term meaning a force that strains or deforms. 'Stress' was first used in a biological sense by a Harvard physiologist, Walter B. Cannon in the 1920's (Cannon, 1927). Cannon used the term to describe the effects that emotions have on physiology and health. However, the term "Stress" became popular in a biologic sense beginning with the work of Hans Selye. In 1950 Selye published an influential book summarizing his work studying "The General Adaptation Syndrome" and the diseases of adaptation titled *The Physiology and Pathology of Exposure to Stress* (Selye, 1950). Since then, this concept has become so pervasive, that today the biologic sense of the word is probably the first that comes to most peoples' minds. Awareness of stress and the effects of stress has become so pervasive in our modern society that in June of 1983, Time magazine ran a cover story which referred to stress as "The Epidemic of the 80"s". Awareness of the effects of stress, and even the number of stressors we experience, has escalated since then.

As William Shakespeare once said "Nothing is good or bad but thinking makes it so", which is one reason the effects of stressful situations are so different for different individuals. In today's world our stressors can include but are certainly not limited to: Traffic jams, Deadlines, Eating on the run, An angry spouse, or boss, or parent, Teenagers, Bills to pay, A job, No job, New job, Job changes, Moving, Endless chores, The new baby, Screaming kids, School, Tests, Getting called on in class, Talking in front of an audience, Writing assignments, Illness, Bereavement, Divorce, Poverty, Friends, No friends, Errands and Demands, Demands, and More Demands. And of course we know the Fear of war, Fear of terror, Fear of the unknown...and the Threat of pandemic disease such as Avian Influenza and even Ebola.

Some Stressors of Animal Production. Most of my career has been involved in studying the stressors involved in turkey production. Some of those are: Hatching, Catching, Handling, Beak and toe trimming, Vaccination, Transportation, Cold Stress, Open field stress, Heat stress, People, Ammonia, Dust, Endotoxin, Disease, Malnutrition, Coming into lay, Social hierarchy, and Overcrowding. There are certainly more and you can also add to the list the other stressors specific to mammalian animal production including Birthing, Weaning, and Lactation. Every grower and production manager can add to this list. A turkey grower in Kansas wrote to me that he thought running the housekeeping tiller in his brooding house was really scaring his poults. He believes that since he stopped tilling, his birds have had less osteomyelitis, a disease attributed to stress. Coincidence? Maybe, but if you add together all of the little things you can

do to make animals more comfortable and less fearful, you can make a big difference in their health. In the U.S. one of the greatest stressors on turkey poults is the move from one house to another in a multi-stage system. We need to find alternatives to these types of management practices that can really impact not only gut health and litter conditions, but also production values, immunity, and food safety.

The Brain-Gut Connection. Recent research has reinforced the long-held anecdotal view that stressors have a significant effect on gastrointestinal function and health. Stress models in laboratory animals, including immobilization stress, thermal injury, and early maternal deprivation suggest that stressors can cause gastric ulcers, affect bacterial populations, alter gastrointestinal motility and ion secretion, and increase intestinal permeability and bacterial translocation (Caso et al., 2008). Recently stress has been shown to affect the microbial ecology of the gut. Exposure of young male mice to an aggressive older mouse results in disruption of the colonic microbiota and importantly, decreases the relative proportions of the genus *Lactobacillus* that are closely associated with the mucosa (Bailey et al., 2011; Galley et al., 2014; Galley and Bailey, 2014). This effect may be especially important to animal production because social stress is common in the production environment and certain strains of *Lactobacilli* have been repeatedly associated with both human and animal intestinal health (Villena and Kitazawa, 2014; Ritchie and Romanuk, 2012).

In broiler chickens, acute stressors including 24-hour heat stress and 24-hour feed withdrawal, resulted in changes in the normal intestinal microbiota and epithelial structure that increased *Salmonella* Enteritidis attachment (Burkholder et al., 2008). Chronic heat stress was also shown to decrease performance and induce intestinal inflammation in chickens infected with *Salmonella* Enteritidis (Quinteiro-Filho et al., 2012). Recently, overcrowding stress (was shown to decrease macrophage activity, induce enteritis and increase *Salmonella* Enteritidis invasion of the liver in broiler chickens (Gomes et al., 2014).

The gut has been shown to develop and grow rapidly during the early period post hatch in poultry species (Uni et al., 2000). Antibiotic growth promoters (AGP's) have been an economical means to improve this development and thus modulate the gut microbiome, increase growth and help prevent disease (Dibner and Richards, 2005). In addition, production stressors have been shown to be a major factor in the inflammatory changes in gut architecture and ecology that are moderated through the use of AGPs.

The disappearance of antibiotic growth promoters? Public pressure, as well as regulatory pressures to limit antibiotic usage in livestock and recent international marketing agreements that prohibit treating poultry with antibiotics, are limiting the disease-fighting tools available to poultry and livestock producers. There is a need to evaluate potential antibiotic alternatives to both increase production and improve disease resistance in high intensity food animal agriculture. Nutritional approaches to counteract the debilitating effects of stress on the gastrointestinal system may provide producers with such alternatives. Improving the disease resistance of animals grown without antibiotics can benefit the animals' health, potentially increasing production efficiency and food safety.

Growth promoting and therapeutic antibiotics have been used to compensate for the high levels of stress that can be present in intensive animal production, because stress can lower resistance to many of the microorganisms always present in the environment. The broiler chicken has been bred for centuries to become an animal that is now well adapted to intensive agriculture and has been shown to grow productively under commercial conditions without antibiotic growth promoters (Wierup and Wegener, 2006). However the food animals that may be more difficult to produce without antibiotics are those that are more reactive under modern

production conditions and have the highest response to production stressors, such as turkeys, veal calves, and weanling pigs.

While growth promoting antibiotics are thought to function mainly by changing the intestinal bacterial flora and affecting gut development, another mechanism by which they may improve production values is through their ability to decrease subclinical disease with the opportunistic pathogens that are present in the environment, such as *E. coli*. The stressors of intensive animal production can lead to changes in the immune response that make animals susceptible to these pathogens and thus lead to disease. Our research program, using an *E. coli* challenge model, has allowed us to study the effects of different kinds of stress on disease and develop nutritional strategies for increasing both disease resistance and production values in turkeys and broiler chickens. While we have explored a number of feed additives for the purpose of replacing antibiotics, including vitamins, adaptogenic herbs, prebiotics and probiotics, yeast (*Saccharomyces cerevisiae*) feed additives have had the most consistent effects in experimental challenges using various stressors and *E. coli* challenge in both chickens and turkeys.

Yeast Feed Additives. Yeast cell walls are potential immunomodulators that may serve as alternatives to antibiotics for both growth promotion and disease resistance in animal production and appear to function as prebiotics in maintaining gut health. Brewer's yeast (*Saccharomyces cerevisiae)* extracts, which are by products of beer manufacturing, have been added to animal feeds for many years for their nutritional content. Brewers dried yeast has been used as a source of both mannan-oligosaccharides (MOS) and ß-1,3/1,6-glucan by a number of companies providing antibiotic-replacement products for animal production. Yeast extracts or yeast cell wall components have been shown to improve gut health, immunity, and production values of turkey poults (Bradley *et al.,* 1994; Fritts and Waldroup, 2003; Huff et al., 2011), broiler chicks (Zhang *et al.* 2005, Huff *et al.,* 2006a: Morales-Lopez and Brufau, 2013; Shao et al., 2013), weanling pigs (Kiarie et al., 2011; Weedman et al., 2011; Upadrasta et al., 2013) and dairy calves (Magalhaes et al., 2008; Eicher et al., 2010; Brewer et al., 2014). Yeast extract products including ß-1,3/1,6-glucan and MOS are generally recognized as safe (GRAS) by the FDA for use as food and feed additives. While many proprietary processes are used to prepare these products, they will be largely referred to in the following discussion as yeast extracts (YE). The following review will demonstrate the effects of YE supplementation in stress challenges of turkeys.

USDA/ARS RESEARCH USING YEAST EXTRACT PRODUCTS IN TURKEY POULTS

The objectives of the following 3 studies were to 1. Evaluate the effects of YE on gut maturation of turkey poults, 2. To determine if YE can protect turkey poults from the effects of cold stress and *E. coli* infection, and 3. Determine if YE can protect turkey poults from the effects of transport stress and *E. coli* infection.

Objective 1. The effects of YE on gut maturation of turkey poults.

Objective 1 Methods. Two replicate trials were undertaken using 180 day-of hatch poults obtained from a commercial hatchery and placed in battery brooders. In both trials poults were fed an unmedicated turkey starter diet or the same diet supplemented with 1lb/ton (@ 500 g/ton) or 2 lb/ton (@ 1000 g/ton) of yeast extract (YE). There were 3 randomized pens with 10 birds/pen in each treatment. For objective 1, a sample of 9 birds/treatment/day were evaluated for gut morphology from both trials. These birds were euthanized and weighed on d 7 and 21, and a 2-cm section was collected from the mid-point of the duodenum, jejunum and ileum of each bird and fixed in a 10% formalin solution for 72 h and then stained. Twenty measurements

of each gut parameter (villus height, villus surface area, lamina propria thickness, crypt depth and the density of neutral, sialomucins, and sulfomucin goblet cells) were taken per section per poult.

Objective 1 Results. In both trials, week 1 body weight was increased by YE supplementation (Figure 1). Yeast extract supplementation influenced intestinal morphology differently based on gut location. Ileum villus height, surface area, lamina propria thickness and crypt depth were enhanced (Table 1) as well as neutral, sialomucin and sulfomucin goblet cell density (Figure 2) with YE treatments on d 7 and 21 ($P<0.05$) and in a dose dependent manner for many of the parameters evaluated. Examples of the differences in ileum goblet cell density between control fed and the YE 2 lb/ton treatment are shown in Figure 3. Jejunum results were mixed. Surface area and crypt depth and sialomucins and sulfomucin goblet cells were consistently higher for the 2 lb/ton YE groups compared to controls on d 7 and 21. Duodenum villus height, surface area, and goblet cell density were higher for the 2 lb/ton YE groups on day 7, however intestinal morphology of the duodenum was not different between the controls and treated birds treated on day 21. Jejunum and duodenum data is not presented here, but has been reported (Solis de los Santos *et al.*, 2007).

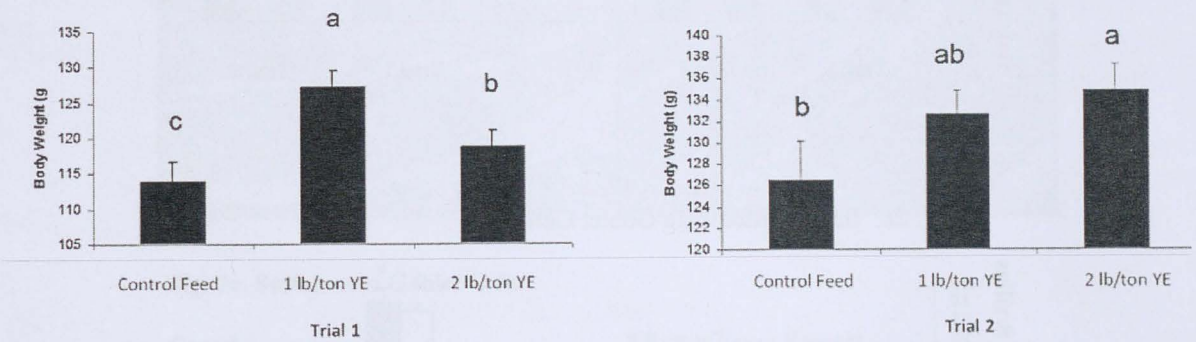


Figure 1. Week 1 body weights of control and yeast extract (YE) supplemented turkey poults. (Data reprinted with the persmission of *Poultry Science*.

Fig. 2a. Ileum Neutral Goblet Cells

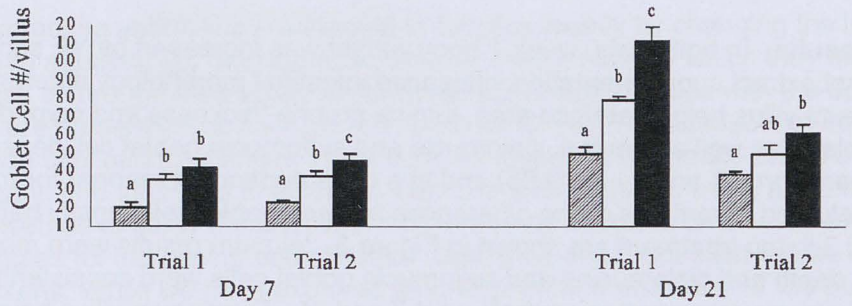


Fig. 2b. Ileum Sialomucin Goblet Cells

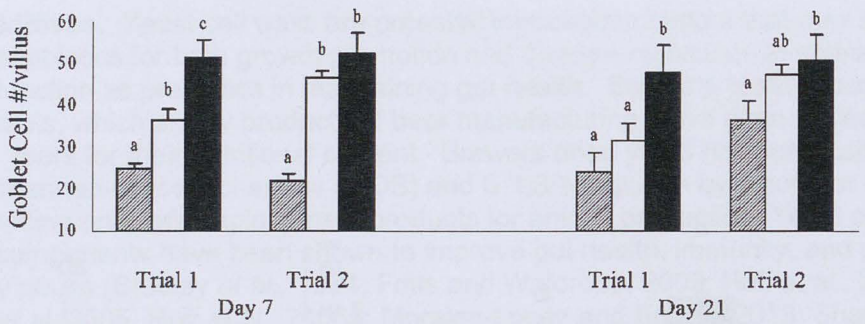


Fig. 2c. Ileum Sulfomucin Goblet Cells

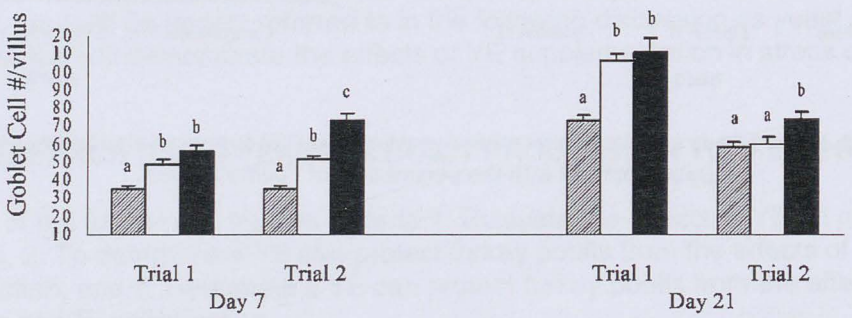


Figure 2. Effect of Yeast Extract™ treatments ▒ control ⌴ 1lb/ton Yeast Extract™ and ■ 2lb/ton Yeast Extract™ on ileum neutral (Fig. 2a.), sialomucins (Fig. 2b.) and sulfomucin (Fig. 2c.) goblet cell density in turkey poults on day 7 and 21. Values are means ± SEM representing cell density in10 well-oriented villi per bird per day of treatment. Means with no common superscript differ ($P \leq 0.05$) between treatments within trials.

Data reprinted with permission of Poultry Science.

Fig. 3a. Neutral Goblet Cells

Control **2 lb/ton Yeast Extract**

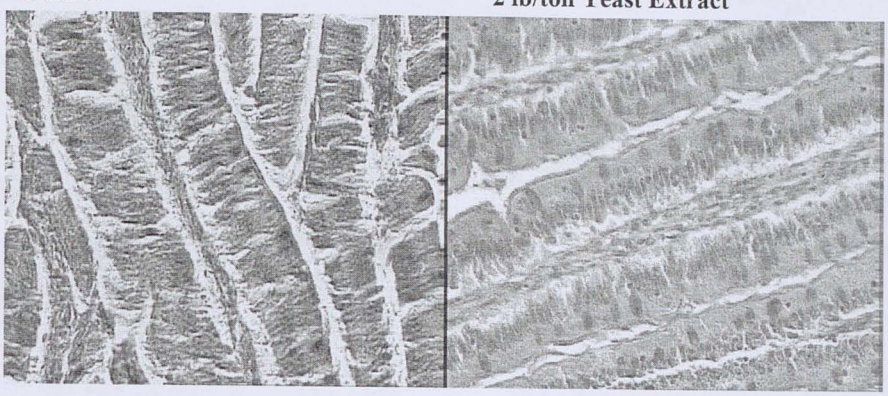


Fig. 3b. Sialomucin Goblet Cells

Control **2 lb/ton Yeast Extract**

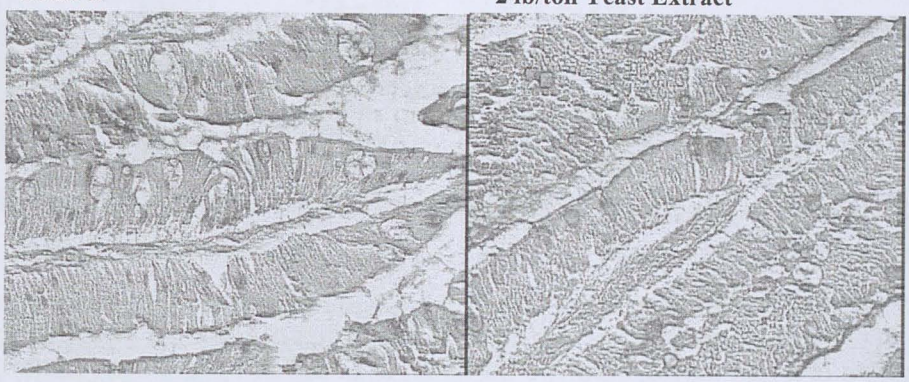


Fig. 3c. Sulfomucin Goblet Cells

Control **2 lb/ton Yeast Extract**

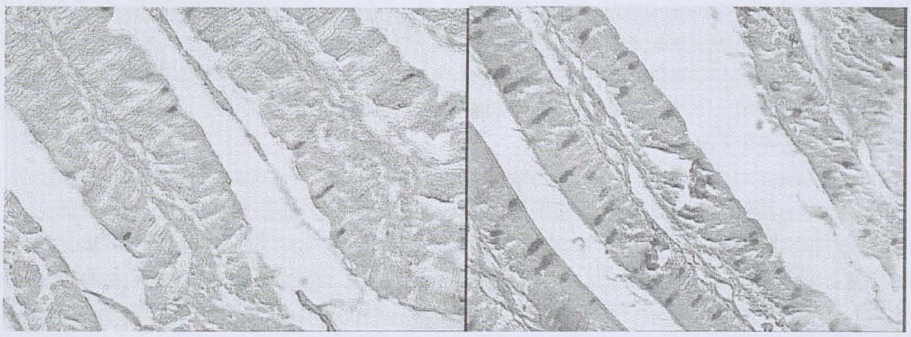


Figure 3. 2 lb/ton of yeast extract increased the number of neutral, acidic (sialomucin and sulfomucin) stained goblet cells in the ileum compared to control treatment on d 7. Similar effect was observed in the ileum on d 21. Neutral goblet cells stained red (Fig. 3a.) with Periodic Acid Schiff staining (PAS). To differentiate acidic goblet cells a combination of high iron diamine-alcian blue (HID-AB) staining was used. With the HID-staining, sialomucins goblet cells stain blue (Fig 3b) whereas sulfated goblet cells stain black (Fig. 3c).(Data reprinted with permission of Poultry Science.)

Table 1. Effect of Yeast Extra Treatment on the Ileal Morphology of Poults at 7 and 21 d of age

	Day 7		Day 21	
	Trial 1	Trial 2	Trial 1	Trial 2
Villus Height (μm)				
Control	310.6 ± 22.8 [a]	411.2 ± 39.7 [a]	629.5 ± 55.2 [a]	484.2 ± 31.0 [a]
1lb/ton Yeast Extract	366.7 ± 29.1 [a]	539.2 ± 45.0 [b]	753.8 ± 61.8 [b]	726.0 ± 53.1 [b]
2lb/ton Yeast Extract	453.3 ± 33.6 [b]	620.3 ± 23.5 [b]	799.8 ± 77.8 [b]	662.0 ± 30.0 [b]
Villus Surface Area (μm^2)				
Control	20,770.9 ± 2391.8 [a]	24,430.3 ± 2555.1 [a]	53,798.3 ± 4785.8 [a]	43,113.1 ± 3865.5 [a]
1lb/ton Yeast Extract	27,341.7 ± 2276.4 [ab]	47,356.8 ± 4268.0 [b]	77,685.0 ± 7801.2 [b]	74,668.6 ± 5195.1 [b]
2lb/ton Yeast Extract	32,548.3 ± 2290.3 [b]	51,629.8 ± 2129.5 [b]	78,423.4 ± 6250.1 [b]	72,594.8 ± 4469.9 [b]
Lamina Propria Thickness (μm)				
Control	64.7 ± 3.9 [a]	76.5 ± 4.1 [a]	102.5 ± 7.3 [a]	99.0 ± 8.6 [a]
1lb/ton Yeast Extract	78.8 ± 4.1 [ab]	99.2 ± 5.0 [b]	117.6 ± 8.9 [a]	142.0 ± 6.0 [b]
2lb/ton Yeast Extract	94.0 ± 9.8 [b]	109.8 ± 5.8 [b]	146.6 ± 7.2 [b]	132.0 ± 7.3 [b]
Crypt Depth (μm)				
Control	97.9 ± 5.9 [a]	110.7 ± 6.8 [a]	136.4 ± 7.2 [a]	111.3 ± 3.0 [a]
1lb/ton Yeast Extract	115.2 ± 6.7 [b]	156.6 ± 8.3 [b]	173.8 ± 4.2 [b]	175.0 ± 6.2 [b]
2lb/ton Yeast Extract	130.0 ± 3.6 [b]	181.0 ± 7.6 [c]	191.1 ± 7.6 [b]	196.7 ± 4.2 [c]

Means ± SEM representing 9 birds per group and 20 measurements per parameter per bird.
[a,b,c] Significant $P < 0.05$ between treatments (vertical) Data reprinted with permission of Poultry Science.

Objective 2. Effects of yeast extract on resistance to cold stress and *E. coli* respiratory challenge.

Objective 2 Methods. Birds from Trial 1 were challenged by exposure to intermittent cold stress (12-16°C) during weeks 1-3 (Table 2), and inoculation of eye and nose by coarse spray of a 10^8 cfu culture of a non-motile, serotype O2 strain of *E. coli* at 1 wk of age. Controls were neither stressed nor inoculated. Birds were bled and necropsied at 3 wk of age.

Objective 2 Results. One lb/ton YE significantly increased week 1 body weight of both control and cold stressed birds relative to birds fed unsupplemented feed, while 2 lb/ton increased week 1 body weight of cold stressed birds only (Figure 4). Week 2 body weights of both control and cold stressed birds were increased by 1 lb/ton YE (Figure 5). Week 3 body weights of cold stressed birds were protected by both 1 lb/ton and 2 lb/ton YE (Figure 6). The reduction seen in feed conversion efficiency due to cold stress was prevented by both levels of YE supplementation (Figure 7).

Table 2. Intermittent cold stress schedule.

Age of bird (Days)	Duration of cold stress (Hours)	Temperature[1] °C
6	1	15.1 ± 2.2
7	2	13.3 ± 2.0
9	3	13.0 ± 1.6
11	7	13.1 ± 1.7
19	8	13.2 ± 1.0

[1]Mean value of temperature at beginning and end of cold stress ± SEM.

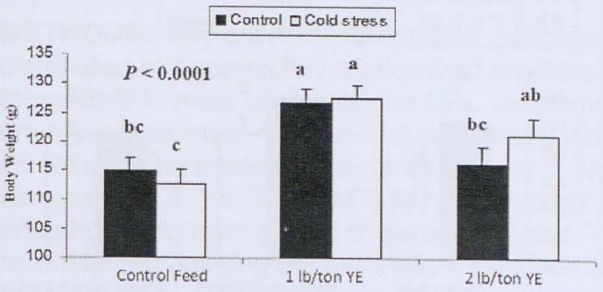


Figure 4. Week 1 body weight

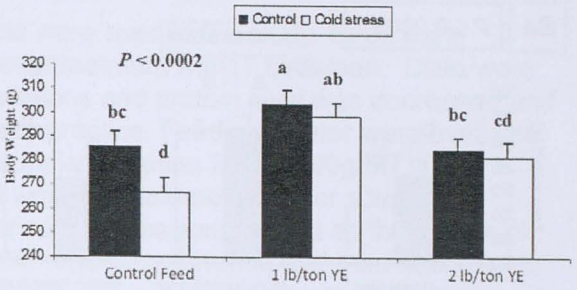


Figure 5. Week 2 body weight

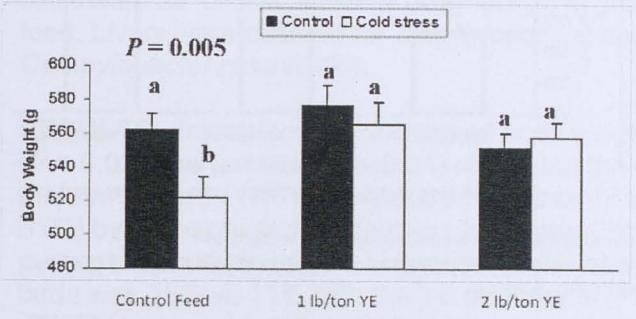


Figure 6. Week 3 body weight

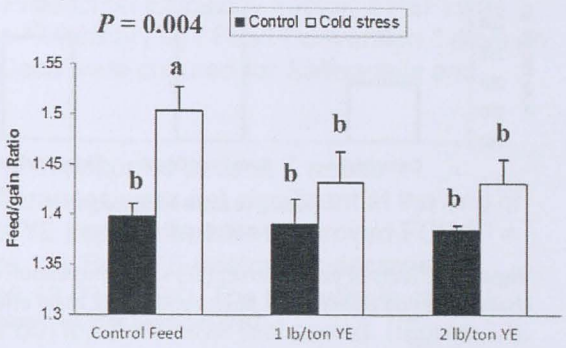


Figure 7. Feed conversion efficiency

Objective 3. Effects of yeast feed supplementation on resistance to *E. coli* challenge followed by transport stress

Objective 3 Methods. Poults from Trial 1 were challenged by air sac injection with 60 cfu of *E. coli* at 1 week of age. At 3 weeks of age these challenged birds were also subjected to transport stress. Birds were placed in coops and driven for 3 hours, then held in the same coops for 9 hours, giving a total of 12 hours of containment without feed or water. Treatment controls were neither stressed nor inoculated. Birds were returned to their original pens and provided feed and water. The next morning nine birds from each experimental group were bled and all birds were necropsied.

Objective 3 Results. Both levels of YE supplementation increased the week 2 and week 3 body weight of challenged birds relative to control fed challenged birds (Figure 8a, 8b). The *E. coli* challenge alone did not significantly affect week 2 body weight of control fed birds (Figure 8a), however following transport stress the week 3 body weight was decreased in the control fed challenged birds (Figure 8b) and this decrease was prevented by both levels of YE. Main effect mean body weights were improved by both levels of supplementation at week 2 and by 1 lb/ton YE at week 3 (Figure 8c, 8d). Feed conversion efficiency was reduced by *E. coli* challenge and transport however there was no effect of YE supplementation (Figure 9). The heterophil/lymphocyte (H/L) ratio was increased by *E. coli* challenge and transport stress. The increase in H/L ratio, which is an indicator of stress, was prevented by both levels of YE supplementation (Figure 10).

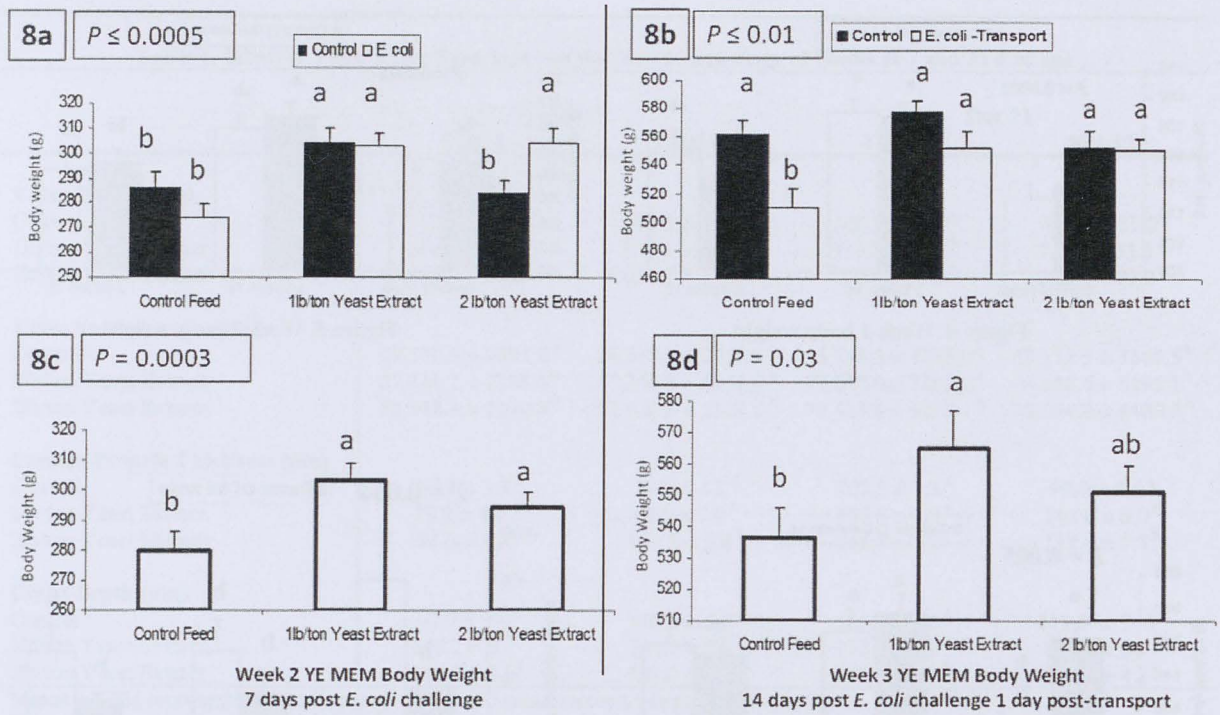


Figure 8. Effects of yeast extract (YE) supplementation on week 2 (8a) and week 3 (8b) body weight of *E. coli* challenged and transported poults and the YE main effect mean (MEM) body weights at week 2 (8c) and week 3 (8d). Data reprinted with permission of Poultry Science.

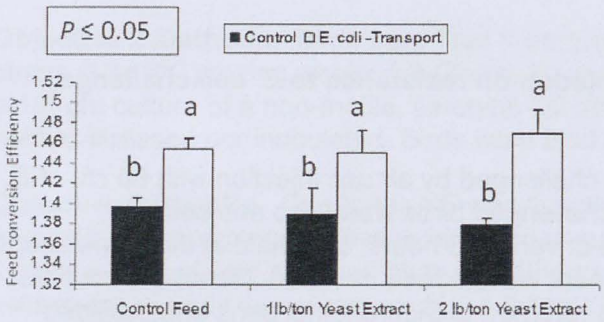


Figure 9. Feed conversion efficiency (total feed consumed / total weight gain)

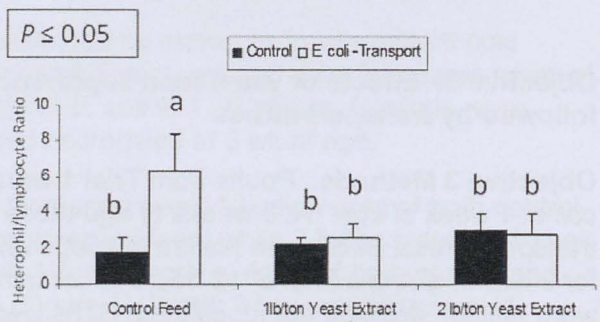


Figure 10. Heterophil/lymphocyte ratio the morning after transport stress.

USDA/ARS RESEARCH USING YEAST EXTRACT PRODUCTS IN MATURE TURKEYS

OBJECTIVE. To determine if YE would prevent the deleterious effects of transport stress and environmental *E. coli* challenge throughout a 16-week grow-out.

RATIONALE. In the United States, turkeys are often grown in a production system that requires moving them to larger barns several times during the lifespan. We have determined that this practice can cause an excessive degree of stress in male turkeys and can result in lower production values and increased disease at the processing plant (Huff et al., 2006b, 2010).

METHODS. Day old male commercial turkey poults were used in the study. Birds were distributed in 4 completely randomized replicate pens/treatment with17 birds/pen. Diets were formulated to meet or exceed the NRC recommendations and protein level was decreased and energy increased in 4 phases as in standard industry practice. Feed and water were available *ad libitum*. The treatments were as follows: 1. No supplementation 2. YE (100g/MT) fed continuously 3. YE (200 g/MT) fed only during first week of placement and for a week encompassing each period of transport stress. Transport stress was defined as the effects of catching and carrying the birds into a transport truck, driving for 3 hours, and then maintaining the birds in new pen and new social groups, without feed or water, for 9 hours. Transport occurred at 6, 12 and 16 weeks of age to mimic industry practice in a 3 stage production system. During the week of transport stress, on alternate days birds were exposed to an environmental challenge with a non-motile, serotype O2 strain of *E. coli* by ocular and intranasal spray with 5 ml of $5x10^8$ cfu/ml. Body weight and feed consumption were recorded weekly and feed efficiency (FE) was calculated. The European Production Efficiency Factor(EPEF) was calculated as follows: EPEF = Body weight in grams * Viability (%) / Feed Conversion * days on feed. Livers were cultured for *Staphylococcus* and Ceca were cultured for *Salmonella* and *Campylobacter* colonization.

RESULTS. Intermittent YE increased body weight of transported birds at 7 weeks ($P = 0.02$) and 9 weeks ($P = 0.04$) of age but the differences were not significant at the end of the trial (Figure 11). The continuous and intermittent YE supplementation improved FCE ($P = 0.07$) by 21 points and 36 points respectively (Figure 12). Both YE treatments decreased percent mortality from 29% in control to 11% at week 13 ($P = 0.04$). The mortality in control birds was 15% vs 11% with the YE treatments (P>0.05) for the overall trial period. (Figure 13). EPEF increased by 35 and 50 points respectively for continuous and intermittent YE supplementation (Figure 14).

CONCLUSIONS. Yeast extract supplementation to turkey diets could ameliorate some of the deleterious effects of stress and may improve feed conversion efficiency in stressed birds. Intermittent YE supplementation at 200g/MT during periods of stress may provide better results as a turkey feed supplement than continuous supplementation at 100 g/MT.

Figure 11. Effect of Yeast Extract on 16 week BW

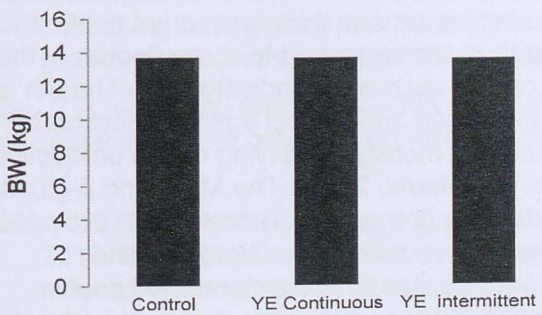


Figure 12. Effect of Yeast Extract on Feed Conversion Efficiency

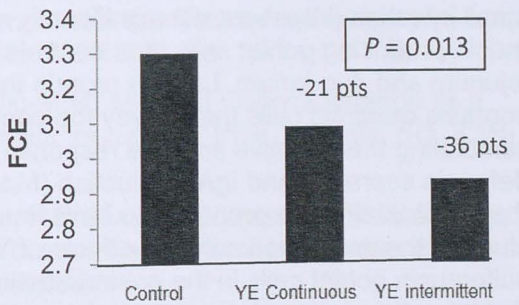


Figure 13. Effect of Yeast Extract on total mortality

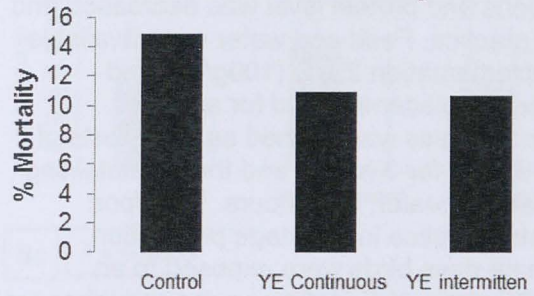
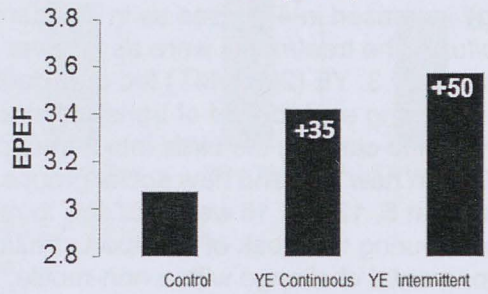


Figure 14. Effect of Yeast Extract on European Production Efficiency Factor (EPEF)



DISCUSSION

These results illustrate the dramatic effects that stress and subclinical disease challenge can have on production values and suggest that feed supplementation with YE may be effective in preventing the production losses due to subclinical *E. coli* infection in turkey poults. The primary method of controlling *E. coli* infections in poultry is through the use of therapeutic antibiotics. Since the 1950's sub-therapeutic levels of antibiotics have been used to improve production values, particularly feed conversion efficiency, presumably by modifying gut bacterial ecology and development (Dibner and Richards, 2005). However, sub-therapeutic antibiotics are also credited with decreasing morbidity and mortality from both clinical and subclinical infections with opportunistic pathogens such as *E. coli* (Gersema and Helling, 1986) and have allowed the development of increasingly intensive confinement animal production.

The ability of YE to accelerate gut maturation may be a mechanism for the protective effects seen in these studies. Enhanced gut development was accompanied by improvement in body weight pre-challenge and may also have influenced the increases seen in production values in subsequent stress challenges. Yeast extract improved body weight in both cold stress and transport stress challenges and poults that were exposed to cold stress had improved feed conversion efficiency. The stress response, as determined by the heterophil/ lymphocyte ratio, was lowered in YE supplemented poults that were subjected to transport stress.

Enhancement of gut maturation was more pronounced in the ileum than in other portions of the small intestine. Yeast extract significantly enhanced lamina propria thickness, crypt depth and mucin producing goblet cells over controls consistently in the ileum and to some degree in the jejunum and duodenum. Lamina propria thickness can be used as an indicator of gut health as it contains dendritic cells that survey the contents of the lumen and protect against infection by stimulating the adaptive immune response, increasing gut motility, modifying mucin production, defensin secretion and IgA production (Macpherson and Harris, 2004). The MOS and β-glucans that YE contains are proposed to have immunomodulating properties. These results provide clues to the immunostimulatory effects of YE as the number of neutral, sialomucin and sulfomucin goblet cells in the gastrointestinal tract were increased in supplemented poults. Goblet cells secrete glycoprotein compounds known as mucins which form the mucus layer that protects the intestinal surface from the invasion of enteric bacteria, toxins, and some dietary components that may damage the mucosa (Specian and Oliver, 1991). These studies suggest that supplementation of turkey diets with YE can enhance gut development and may be valuable for preventing the production losses, particularly increased feed conversion ratio, that

are due to stress. Further details of the methods used and additional data are available (Solis de los Santos *et al.,* 2007; Huff *et al.,* 2007, 2013).

ACKNOWLEDGEMENTS

The data presented herein was co-authored by F. Solis de los Santos, D.J. Donoghue, and B. Packialakshmi (Department of Poultry Science, University of Arkansas, Fayetteville, AR), W.E. Huff, N.C. Rath, and A.M. Donoghue (USDA, Agricultural Research Service, Poultry Production and Product Safety Research Unit, Fayetteville, AR), M.B. Farnell (Department of Poultry Science, Texas A&M University, College Station, TX), and S. Jalukar and J. Oppy (Vi-COR, Mason City IA).

REFERENCES

Bailey, M.T., S.E. Dowd, J.D. Galley, A.R. Hufnagle, R.G. Allen, and M. Lyte, 2011. Exposure to a social stressor alters the structure of the intestinal microbiota: implications for stressor-induced immunomodulation. Brain, Behav. Immun. 25:397-407.

Bradley, G.L., T.F. Savage, and K.I. Timm, 1994. The effects of supplementing diets with *Saccharomyces cerevisiae* var. boulardii on male poult performance and ileal morphology. Poult. Sci. 73: 66-70.

Brewer, M.T., K.L. Anderson, I. Yoon, M.F. Scott, and S.A. Carlson, 2014. Amelioration of salmonellosis in pre-weaned dairy calves fed *Saccharomyces cerevisiae* fermentation products in feed and milk replacer. Vet. Microbiol.172:248-255.

Burkholder, K.M., K.L. Thompson, M.E. Einstein, T.J. Applegate, and J.A. Patterson, 2008. Influence of stressors on normal intestinal microbiota, intestinal morphology, and susceptibility to *Salmonella* Enteritidis colonization in broilers. Poult. Sci. 87:1734-1741.

Cannon, W.B., 1927. Bodily Changes in Pain, Hunger, Fear, and Rage: An Account of Recent Researches into the Function of Emotional Excitement. D. Appleton and Company. New York and London.

Caso, J.R., J.C. Leza, and L. Menchen, 2008. The effects of physical and psychological stress on the gastro-intestinal tract: lessons from animal models. Curr. Mol. Med. 8:299-312.

Dibner, J. J., and J. D. Richards, 2005. Antibiotic growth promoters in agriculture: history and mode of action. Poult. Sci. 84:634-643.

Eicher, S.D., I.V. Wesley, V.K. Sharma, and T.K. Johnson, 2010. Yeast cell-wall products containing beta-glucan plus ascorbic acid affect neonatal Bos Taurus calf leukocytes and growth after a transport stressor. J. Anim. Sci. 88:1195-1203.

Fritts, C.A. and P.W. Waldroup, 2003. Evaluation of Bio-Mos® Mannan Oligosaccharide as a replacement for growth promoting antibiotics in diets for turkeys. Inter. J. Poult. Sci. 2:19-22.

Galley, J.D., M.C. Nelson, Z. Yu, S.E. Dowd, J. Walter, P.S. Kumar, M. Lyte, and M.T. Bailery, 2014. Exposure to a social stressor disrupts the community structure of the colonic mucosa-associated microbiota. BMC Microbiol. 14:189. http://www.biomedcentral.com/1471-2180/14/189

Galley, J.D. and M.T. Bailey, 2014. Impact of stressor exposure on the interplay between commensal microbiota and host inflammation. Gut Microbes Apr. 1;5(3). [Epub ahead of print]

Gersema, L.M., and D.K. Helling. 1986. The use of subtherapeutic antibiotics in animal feed and its implications on human health. Drug Intell. Clin. Pharm. 20:214-218.

Gomes, A.V.S., W.M. Quintero-Filho, A. Ribeiro, V. Ferraz-de-Paula, M.L. Pinheiro, E. Baskeville, A.T. Akamine, C.S. Astolfi-Ferreira, A.J.P. Ferreira, and J. Palermo-Neto, 2014.

Overcrowding stress decreases macrophage activity and increases *Salmonella* Enteritidis invasion in broiler chickens. Avian Path. 43:82-90.

Huff, G.R., W.E. Huff, N.C. Rath, and G. Tellez, 2006a. Limited treatment with Beta-1,3/1,6-glucan improves production values of broiler chickens challenged with *Escherichia coli*. Poult. Sci. 85:613-618.

Huff, G., W. Huff, N. Rath, J. Balog, N.B. Anthony, and K. Nestor. 2006b. Stress-induced colibacillosis and turkey osteomyelitis complex in turkeys selected for increased body weight. Poult. Sci. 85:266-272.

Huff, G.R., W.E. Huff, N.C, Rath, F. Solis de los Santos, M.B. Farnell, and A.M. Donoghue, 2007. Influence of hen age on the response of turkey poults to cold stress, *Escherichia coli* challenge, and treatment with a yeast extract antibiotic alternative. Poult. Sci. 86:636-642.

Huff, G.R., W.E. Huff, M.B. Farnell, N.C. Rath, F. Solis de los Santos, and A.M. Donoghue, 2010. Bacterial clearance, heterophil function, and hematological parameters of transport-stressed turkey poults supplemented with dietary yeast extract. Poult. Sci. 89:447-456.

Huff, G.R., V. Dutta, W.E. Huff, and N.C. Rath, 2011. Effects of dietary yeast extract on turkey stress response and heterophil oxidative burst activity. Brit. Poult. Sci. 52:446-455.

Huff, G.R., W. E. Huff, S. Jalukar, J. Oppy, N. C. Rath, and B. Packialakshmi, 2013. The effects of yeast feed supplementation on turkey performance and pathogen colonization in a transport stress/*Escherichia coli* challenge. Poult. Sci. 92:655–662

Kiarie, E., S. Bhandari, M. Scott, D.O. Krause, and C.M. Nyachoti, 2011. Growth performance and gastrointestinal microbial ecology responses of piglets receiving *Saccaromyces cerevisiae* fermentation products after an oral challenge with *Escherichia coli* (K88), 2011.

Magalhaes, V.J., F. Susca, F.S. Lima, A.F. Branco, I. Yoon, and J.E. Santos, 2008. Effect of feeding yeast culture on performance, health, and immunocompetence of dairy calves. J. Dairy Sci. 91:1497-1509.

Morales-Lopez, R., and J. Brufau, 2013. Immune-modulatory effects of dietaty *Saccharomyces cerevisiae* cell wall in broiler chickens inoculated with *Escherichia coli* lipopolysaccharide. Brit. Poult. Sci. 54:247-251.

Quinteiro-Filho, W.M., A.V.S. Gomes, M.L. Pinheiro, A. Ribeiro, V. Ferraz-de-Paula, C.S. Astolfi-Ferreira, A.J.P. Ferreira, and J. Palermo-Neto, 2012. Heat stress impairs performance and induces intestinal inflammation in broiler chickens infected with *Salmonella* Enteritidis. Avian Path. 41:421-427.

Ritchie, M.L. and T.N. Romanuk, 2012. A meta-analysis of probiotic efficacy for gastrointestinal diseases. PLoS ONE 7(4):e34938. Doi:1371/journal.pone.0034938.

Selye, H., 1950. The Physiology and Pathology of Exposure to Stress. A treatise based on the Concepts of the General-Adaptation-Syndrome and the Diseases of Adaptation. Acta, Inc., Montreal, Canada.

Shao, Y., Y. Guo, and Z. Wang, 2013. B-1,3/1,6-Glucan alleviated intestinal mucosal barrier impairment of broiler chickens challenged with *Salmonella entérica* serovar Typhimurium. Poult. Sci. 92:1764-1773.

Solis de los Santos, F., A.M. Donoghue, M.B. Farnell, G.R. Huff, W.E. Huff and D.J. Donoghue, 2007. Gastrointestinal maturation is accelerated in turkey poults supplemented with a mannan-oligosaccharide yeast extract (Alphamune™). Poult. Sci. 86: 921-930.

Specian and Oliver, 1991. Functional biology of intestinal goblet cells. Am. J. Physiol. 260 (Cell Physiol.29):C183:C193.

Uni, Z., A. Geyra, H. Ben-Hur, and D. Sklan, 2000. Small intestinal development in the young chick: Crypt formation and enterocyte proliferation and migration. Brit. Poult. Sci. 41:544-551.

Upadrasta, A., L. O'Sullivan, O. O'Sullivan, N. Sexton, P.G. Lawlor, C. Hill, G.F. Fitzgerald, C. Stanton, and R. P. Ross, 2013. The effect of dietary supplementation with spent cider yeast on the swine distal gut microbiome. PLoS ONE 8 (10): e75714. doi:10.1371/journal.pone.0075714

Villena, J. and H. Kitazawa, 2014. Modulation of intestinal TLR4-inflammatory signaling pathways by probiotic microorganisms: lessons learned from *Lactobacillu jensenii* TL2937. Frontiers Immun. Vol. 4. Article 512. www.frontiersin.org

Weedman, S.M., M.H. Rostagno, J.A. Patterson, I. Yoon, G. Fitzner, and S.D. Eicher, 2011. Yeast culture supplement during nursing and transport affects immunity and intestinal microbial ecology of weanling pigs. J. Anim. Sci. 89:1908-1921.

Wierup, M and H.C. Wegener, 2006. Termination of AGP use and effect on subsequent production of broiler chickens in Sweden and Denmark during a 25-year period. *In:* Antimicrobial Growth Promoters. Where do we go from here? D. Barug, J. de Jong, A.K. Kies, and M.W.A. Verstegen, eds. Wageningen Academic Publishers. The Netherlands.

Zhang, A.W., B.D. Lee, S.K. Lee, K.W. Lee, G.H. AN, K.B. Song, and C.H. Lee, 2005. Effects of yeast (*Saccharomyces cerevisiae)* cell components on growth performance, meat quality, and ileal mucosa development of broiler chicks. Poult. Sci. 84:1015-1021.

Notes